# EGG BIOSCIENCE AND BIOTECHNOLOGY

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new β-fold motif, the β-prism. Circular dichroism spectra in the far-UV region at room temperature resembled random-coil characteristics, while in the near-UV region, small positive peaks were observed. Unfolding at 67.5–70°C was observed by nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC).

#### *1.3.2.2. Lipids in Albumen*

Albumen contains very low (0.03% w/w) lipid content. The main fatty acids in albumen lipids are palmitic, oleic, linoleic, arachidonic, and stearic acids (Table 1.7) (Watkins et al. 2003).

#### *1.3.2.3. Carbohydrates in Albumen*

Glucose is the main "free" sugar, constituting a ~0.8–1% by weight of albumen. It is usually removed by fermentation prior to drying of egg white to prevent browning caused by Maillard reaction. Carbohydrate is also present in the form of *N*-linked or *O*-linked oligosaccharides conjugated to albumen proteins or glycoproteins (Koketsu 1997).

#### *1.3.2.4. Minerals in Albumen*

As shown in Table 1.8, the major minerals in egg white are sulfur, potassium, sodium, and chlorine; phosphorus, calcium, and magnesium are found in lower quantities, as are various other trace minerals (Sugino et al. 1997b; Watkins 1995).

Kilic et al. (2002) determined levels of some minerals in white and yolk of eggs sampled from villages or farms in Ankara, Turkey. In general, higher levels of minerals such as Cu, Zn, Mg, Ca, and Fe were found in the white from village eggs than from farm eggs. Jacobs et al. (1993) found that 56% of total Se content was associated with ovalbumins 1 and 2 (ca 500 ng/g). Of the proteins identified, flavoprotein had the highest Se content (1800 ng/g). Selenium content of other proteins ranged from 359 to 1094 ng/g. Aydin et al. (2001) reported that eggs from CLA-fed hens had greater Fe, Ca, and Zn concentrations and lower Mg, Na, and Cl concentrations in albumen relative to those from hens fed corn oil.

#### *1.3.2.5. Vitamins in Albumen*

Albumen does not contain fat-soluble vitamins, but does contain significant proportions of the water-soluble vitamins in egg, including biotin, niacin, and riboflavin (Table 1.8) (Watkins 1995). Many of the vitamins are present in a bound form with proteins in albumen, as described in Section 1.3.2.1. However, albumen and egg in general are devoid of vitamin C.

### 1.3.3. Egg Yolk

Egg yolk contains ~50% solids; the major constituents of the solid matter are lipids (~65–70% dry basis) and proteins (~30% dry basis) (Table 1.2). The

composition of the solid matter in vitelline membrane differs from the yolk itself; it is higher in protein (87%) and carbohydrate (10%) than lipids (3%). Poser et al. (2004) found no significant effect of chicken age or storage on protein composition of the vitelline membrane. Lin and Lee (1996) studied relationships between hen age (39, 62, or 93 weeks) and egg composition, and reported that eggs laid by 93-week-old hens were ~18% heavier than those laid by 39-week-old hens, and that the yolk weight (expressed as grams per egg) also increased with hen age.

Egg yolk can be separated into plasma (supernatant) and granule fractions (precipitate) by centrifugation. The plasma makes up ~78% of total liquid yolk; it contains ~51% solids, which consist primarily of lipid (~80%), 2% ash, and 18% nonlipid material that is mostly protein (Li-Chan et al. 1995). Granules, on the other hand, contain ~34% lipid, 60% protein, and 5% ash on a moisture-free basis (Li-Chan et al. 1995). Anton and Gandemer (1997) reported that granules of egg yolk contained about half the lipids and cholesterol and about double the proteins of yolk and plasma. Yolk and granules require a minimum ionic strength of 0.3 *M* NaCl to become solubilized at pH 7.0, whereas plasma is solubilized at any ionic strength.

The composition and content of yolk may be influenced by the diet of the laying hen. Yolk weights and egg weights increased significantly after feeding 10,000- and 20,000-ppm α-tocopheryl acetate (Engelmann et al. 2001). Novak and Scheideler (2001) reported that yolk solids content is significantly increased by Ca supplementation ($p < .03$) as well as by flaxseed supplementation ($p < .06$) compared to a corn–soy control group. Kim et al. (2006) showed that addition of activated charcoal with wood vinegar in layer diet resulted in improved yolk index and yolk color, as well as higher eggshell breaking strength and HU values. Hwangbo et al. (2006) reported that cheese byproduct beneficially improved the fatty acid composition of concern to human health in the egg yolk without adverse effects on egg quality. Triglyceride, cholesterol, and low-density lipoprotein levels in egg yolk were significantly reduced by supplementing the hen's diet with red mold rice (Wang and Pan 2003), but neither plasma lipoprotein composition nor yolk cholesterol content was affected by dietary α-tocopherol and corn oil (Shafey et al. 1999).

Barron et al. (1999) reported that yolk-directed very low-density lipoprotein (VLDLy) concentration in plasma decreased in hens undergoing ovarian regression owing to food and light deprivation, prolactin treatment, or overfeeding, but declined more rapidly in food and light-deprived hens. Lien et al. (2003) reported significant effects of cod liver oil and/or chromium picolinate on the serum traits and egg yolk fatty acids and cholesterol content in laying hens. Lien et al. (2004) found that the VLDL content was markedly reduced while HDL content was significantly increased by Cu and Cr supplementation of the diet of White Leghorn layers hens.

#### *1.3.3.1. Proteins in Yolk*

Egg yolk contains ~16% proteins, consisting of proteins in solution referred to as *livetins*, and lipoprotein particles including high-density lipoproteins (HDL), low-density lipproteins (LDL), and very low-density lipoproteins (VLDL). The total amino acid composition of yolk is presented in Table 1.6, and the major protein components are shown in Table 1.10.

In addition to their nutritional and functional properties, various biological activities have more recently been attributed to the yolk proteins (Mine and Kovacs-Nolan 2006). These include antimicrobial activity, antiadhesive properties, and antioxidant properties. Furthermore, some of the bioactive properties have been ascribed to peptides generated by partial hydrolysis of yolk proteins. For example, Park et al. (2001) purified and characterized antioxidative peptides obtained by hydrolysis of lecithin-free egg yolk by the commercial enzyme Alcalase (subtilism Carlsberg, EC3.4.21.62). Two different peptides exhibiting strong antioxidative activity were composed of 10 and 15 amino acid residues, and both contained a leucine residue at their *N*-terminal positions.

##### *1.3.3.1.1. Low-Density Lipoprotein (LDL) or Lipovitellenins*

Low-density lipoprotein (LDL) represents two-thirds of the yolk solids, and is believed to be responsible for the functional properties of yolk, particularly its emulsifying ability. The low density (0.98) of LDL is attributed to its much higher lipid content compared to protein content (Burley and Vadehra 1989; Anton et al. 2003) (Table 1.11), leading to the suggestion that it should in fact be classified as VLDL.

**TABLE 1.10. Proteins and Lipids in Egg Yolk**

| Constituent | Major Components | Relative % |
|---|---|---|
| Proteins[a] | Apovitellenin I–VI | 37.3 |
| | Lipovitellin apoproteins | |
| | α-Lipovitellin | 26.7 |
| | β-Lipovitellin | 13.3 |
| | Livetins | |
| | α-Livetin (serum albumin) | 2.7 |
| | β-Livetin (α-2-glycoprotein) | 4.0 |
| | γ-Livetin (γ-globulin) | 2.7 |
| | Phosvitin | 13.3 |
| | Biotin binding protein | Trace |
| Lipids[b] | Triglyceride | 65 |
| | Phosphatidylcholine | 26 |
| | Phosphatidylethanolamine | 3.8 |
| | Lysophosphatidylcholine | 0.6 |
| | Cholesterol | 4 |
| | Sphingomyelin | 0.6 |

[a]Modified from Burley and Vadehra (1989).
[b]Adapted from Juneja (1997).

**TABLE 1.11. Composition of Low-Density Lipoproteins from Egg Yolk**

| Component | g/100 g Dry Matter[a] |
|---|---|
| Proteins | 12.0 |
| Total lipids | 86.7 |
| Triglycerides | 62.0 (71%) |
| Phospholipids | 21.5 (25%) |
| Phosphatidylcholine | 18.4 (21%) |
| Phosphatidylethanolamine | 3.0 (3%) |
| Cholesterol | 3.2 (4%) |
| Fatty acid | |
| Palmitic acid (16:0) | (24.7%) |
| Oleic acid (18:1) | (41.1%) |
| Linoleic acid (18:2) | (16.0%) |
| Saturated fatty acids | (34%) |
| Monounsaturated fatty acids | (45%) |
| Polyunsaturated fatty acids | (21%) |

[a]Numbers in parentheses represent percent of total lipid or fatty acid.

*Source*: Adapted from Anton et al. (2003).

Low-density lipoprotein is synthesized and assembled in the liver as a modified blood VLDL, whose main apoprotein is apo B (Burley et al. 1993). Apo B enters yolk by endocytosis, and furthermore yields four of the yolk–lipoprotein apoproteins (apovitellenins III–VI) on enzymatic hydrolysis. Salvante et al. (2001) reported that avian egg production is accompanied by dramatic changes in lipid metabolism, including a marked increase in hepatic production of estrogen-induced, yolk-targeted very low-density lipoprotein (VLDLy). The structure and function of plasma VLDL particles changes from the larger generic, nonlaying VLDL particles that are involved in triglyceride transport throughout the body, to the smaller VLDLy particles that supply the yolk with energy-rich lipid.

Yolk lipoproteins show polydispersity that may partially reflect varying lipid contents or composition, but in addition, there are at least two subfractions known variously as LDL1 and LDL2 (Juneja and Kim 1997), LDF1 and LDF2, or LDP1 and LDP2 (Burley and Vadehra 1989). Six apoproteins (apovitellenins I–VI) have been reported. Apovitellenin I is poorly soluble in the absence of denaturing agents such as urea, guanidine hydrochloride, or sodium dodecylsulfate, and is characterized by a lack of histidyl residues in its amino acid composition. Apovitellenin II is very soluble in salt solutions, and contains *N*-linked polysaccharide chains consisting of glucosamine, hexose, and sialic acid. At least four fractions of apovitellenin II have been reported, with varying properties and composition (Burley and Vadehra 1989). Apovitellenins III–VI have been shown to be derived by proteolysis of apo B (Burley et al. 1993), with possible additional minor apoproteins resulting from varations in the positions of proteolysis.

Mine (1997b) examined structural and functional changes of LDL resulting from enzymatic modification of its phospholipids using phospholipase A2. The $^{31}P$ NMR spectrum and enzyme hydrolysis profiles revealed higher susceptibility of phospholipids in LDL complex to modification compared to phospholipids in small unilamellar vesicles or emulsions, suggesting higher membrane fluidity of LDL and that the interactions of proteins with PLs are not strong. Although the modification of phospholipids in LDL did not affect secondary structure of the apoprotein or the immunologic property of LDL, the emulsions stabilized with modified LDL showed considerable heat stability, possibly through enhancement of phospholipid–protein interactions.

Mine and Bergougnoux (1998) studied adsorption properties of cholesterol-reduced egg yolk low-density lipoprotein (CR-LDL) at oil-in-water (O/W) interfaces. The protein concentration at the interface was greater for emulsions made by CR-LDL than control LDL at pH 7.0 and 3.5, which was attributed to formation of lipoprotein aggregates resulting from cholesterol removal in LDL. Removing the cholesterol from egg yolk LDL caused changes in phospholipid–protein interactions at the interface, which could explain the instability of CR-LDL emulsion. According to Mine (1998), egg yolk LDL micelles break down when the micelles come into contact with the interface, and rearrangement of lipoproteins, cholesterol, and phospholipids take place following adsorption at an O/W interface.

Anton et al. (2003) investigated the effects of hen egg yolk LDL structure and composition on emulsification properties. The extracted LDL consisted of spherical particles with mean diameter of 20–60 nm, and contained ~87% lipid and 12% protein. The solubility of the extracted LDL was >90% under all conditions tested, including a wide range of pH (3–8) and NaCl concentration (0.15–0.55 *M*). Five major apoproteins were extracted from LDL, with molecular weights of 15, 60, 65, 80, and 130 kDa. Of these, the 15-kDa apoprotein appeared have the greatest capacity to absorb at the O/W interface in emulsions, owing its high content of side chains with amphipathic α-helices.

*1.3.3.1.2. High-Density Lipoprotein (HDL) or Lipovitellins*

High-density lipoprotein consists of α- and β-lipovitellins, which differ in amino acid composition as well as bound phosphorus and carbohydrate. The proportion of α- and β-lipovitellins in yolk granules appears to be genetically based. It was reported that β-HDL is resistant to heat (Anton et al. 2000). The protein content of lipovitellins is about 80%, while the lipid content is about 20%, including phospholipids (60% of the lipids, primarily as lecithin), triacylglycerols (40%), and small amounts of cholesterol, sphingomyelin, and other lipids (Burley and Vadehra 1989). Both lipovitellins are glycoconjugates with mannose, galactose, glucosamine, and sialic acid, but α-lipovitellin contains much higher sialic acid content than does β-lipovitellin, explaining its relatively acidic nature (Juneja and Kim 1997). The apoprotein form of lipovitellins, sometimes referred to as *vitelline*, is present in a dimeric form thought

yolk proteins such as low-density lipoprotein, lipovitellins, and phosvitin. Seko et al. (1997) investigated the sialylglycopeptide components of hen egg yolk, localized in the yolk plasma. The amino acid sequence of the peptide moiety of sialyloglycopeptide was determined to be Lys–Val–Ala–Asn–Lys–Thr, with an *N*-linked disialylbiantennary glycan at the Asn residue. Structural information on glycopeptides with neutral and sialylated *N*-glycans has been studied using positive- and negative-ion MSn spectra (Deguchi et al. 2006).

### *1.3.3.2. Lipids in Egg Yolk*

Lipids are the main components (32–36%) of the egg yolk solids. The composition of yolk lipid is about 65% triglyceride, 28–30% phospholipid, and 4–5% cholesterol. The fatty acid composition of lipid in yolk plasma and granules is shown in Table 1.7. The composition of yolk lipids can be affected by various factors including hen age and genotype and changes in the diet of the hens.

Polyunsaturated fatty acid contents were significantly higher for eggs laid by 39-week-old hens compared with older hens, while monounsaturated fatty acid contents were significantly higher for eggs laid by 93-week-old hens (Lin and Lee 1996). The contents of long-chain (20 and 22) omega-6 and omega-3 polyunsaturated fatty acids (PUFA) were 20% and 25%, respectively, higher in egg yolks from 21-week-old hens than 57-week-old hens (Nielsen 1998). Shafey (1996) reported that egg size did not significantly affect yolk lipid or fatty acid concentration. However, lipid levels were lower while linoleic acid level was higher in yolks of eggs from hens older than 47 weeks of age, than in those produced by younger birds. The unsaturated:saturated fatty acid ratio for yolk produced at 39 and 47 weeks of age was greater than that for yolk produced at 31 weeks. However, the monounsaturated:polyunsaturated fatty acid ratio for yolk produced at 27 and 39 weeks of age was lower than that for yolk produced at 51 weeks.

Dziadek et al. (2003a, 2003b) studied the influence of the hen genotype on chemical composition of table eggs, using nine commercial lines of laying hens [Lohmann Brown, Shaver 579, AK (experimental from IZ-OBD Zakrzewo), ISA White, Messa 445, Messa 443, Astra W-1, Astra W-2, and Astra N]. Yolk lipid content ranged from 29.37% in the eggs from AK layers to 31.85% in the eggs from Astra N birds. Triglyceride content in the egg yolk varied from 199.70 mg/g in the Messa 443 group to 236.55 mg/g in the Astra N group of birds. Scheideler et al. (1998) studied the effect of strain and age on egg composition from hens fed diets rich in omega-3 fatty acids, and reported that the percentage of C18:0 and C18:1 fatty acids in the yolk was significantly affected by strain, diet, and strain–diet interaction. Latour et al. (1998) suggested that breeder age influences the utilization of yolk lipid by developing embryos, and that the type of fat (corn oil, poultry fat, or lard) provided in the diet may have an additional influence.

Sim (1998) described the development of "designer eggs" rich in omega-3 PUFA such as α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have been associated with beneficial effects for human health, including reduction of triglyceride level, blood pressure, platelet aggregation, and tumor growth. Many studies have therefore investigated the incorporation of different feed ingredients such as fish oil; vegetable oils, including flaxseed (linseed), soy oil, or canola oil; and microalgae into the diet of hens, in order to optimize the omega-3/omega-6 and PUFA/saturated fatty acid ratios of eggs for human health [e.g., see Scheideler and Froning (1996), An and Kang (1999), Han et al. (1999), Santoso et al. (1999), Yannakopoulos et al. (1999), Daenicke et al. (2000), Herstad et al. (2000), Lewis et al. (2000), Shimizu et al. (2001), Sari et al. (2002), Basmacioglu et al. (2003), Milinsk et al. (2003), Cheng et al. (2004), Zotte et al. (2005), Fredriksson et al. (2006)]. Incorporation of omega-3 PUFA was reported to occur mainly in the *sn*-2 position, particularly of phospholipids (Cossignani et al. 1994), and EPA and DHA contained in eggs were observed to be stable (Oku et al. 1996).

Products enriched with PUFAs are prone to oxidation, and enrichment with antioxidants is necessary in order to prevent the risk of oxidative damage. Grune et al. (2001) suggested supplementation of feed with least 80 IU vitamin E/kg to prevent increase in cytotoxic aldehydic lipid peroxidation during production and storage of omega-3 PUFA-enriched eggs. Gebert et al. (1998) reported that dietary vitamin E resulted in a decrease of PUFA, SFA, and total lipids in fresh yolk lipids, whereas MUFA did not change. Boruta and Niemiec (2005) reported that dietary vitamin E supplement slowed down the process of oxidation of egg yolk fatty acid during storage.

Several studies have also investigated the effect of dietary conjugated linoleic acid (CLA) on the composition of egg yolk lipids. Du et al. (1999) reported that the levels of CLA incorporated into lipid of egg yolk were proportional to levels of CLA in the diet, although more CLA was incorporated in the triglycerol than were phospholipid components, and the incorporation rates of different CLA isomers in different classes of lipids also were significantly different. Furthermore, inclusion of CLA in the diet influenced the metabolism of polyunsaturated fatty acids. Du et al. (2000) reported that the amount of arachidonic acid was decreased by CLA added to linoleic acid– and linolenic acid–rich diets, but EPA and DHA were increased in the linolenic-rich diet, indicating that synthesis or deposition of long-chain *n*-3 fatty acids was accelerated after CLA feeding. However, increases in saturated fatty acids in yolk and decreases in MUFA and PUFA by dietary CLA have also been reported (Schaefer et al. 2001; Hur et al. 2003; Watkins et al. 2003). Szymczyk and Pisulewski (2003) reported that feeding CLA-enriched diets resulted in gradually increasing deposition of CLA isomers ($p < .01$) in egg yolk lipids, while Watkins et al. (2003) suggested that feeding CLAs to hens led to accumulation of isomers in polar and neutral lipids of the egg yolk that migrated into egg albumen.

Aydin et al. (2001) reported that olive oil prevented CLA-induced increases in 16:0 and 18:0 and decrease in 18:1(ω-9) in yolk, and also prevented CLA-induced abnormal changes in the pH of albumen and yolks. Hur et al. (2003) indicated that lipid oxidation of egg yolk during cold storage could be inhibited by dietary CLA due not only to changes in fatty acid composition but also to the high concentration of CLA in egg yolk. Hwangbo et al. (2006) showed that as dietary levels of cheesemaking byproduct increased, linear increases in total CLA and *cis*-9,*trans*-11 CLA contents of egg yolk took place, together with decreases in total saturated fatty acid content. Szymczyk and Pisulewski (2005) reported that dietary vitamin E increased the rate of laying and egg production per hen and may also exert alleviating effects on fatty acid composition of CLA-enriched eggs.

Many other treatments have been reported to affect egg yolk composition. A significant linear reduction was found in plasma and yolk triglycerides (24% and 30%) as the dietary copper content was increased from 0 to 250 mg/kg (Al-Ankari et al. 1998). Reduction of egg cholesterol was obtained by inclusion of red fungus rice containing monacolin K (Wang and Pan 2003). Biswas et al. (2000) reported that levels of egg yolk cholesterol and lipid were significantly reduced by adding Japanese green tea powder in the feed. Li and Ryu (2001) reported that 0.1% wood vinegar tends to improve egg production and significantly increase PUFA (C20:4, C22:6) content in egg yolk. Kim et al. (2001b) found that supplementation of feed with 1.0% Bio-alpha® (a fermented feed containing a range of microorganisms) increased DHA content and reduced cholesterol content of egg yolks significantly compared with controls. Microwave cooking substantially reduced levels of PUFA compared to boiling or frying (Murcia et al. 1999).

Liu et al. (2005b) investigated the composition and quality of lipids in various commercial egg products, including fresh egg, Pidan (preserved egg), tea egg, simmered egg, iron egg (deeply simmered and dried egg), and salted egg. The lipid content ranged from 27% for Pidan to 46% for iron egg. Phospholipid and cholesterol contents were highest in fresh egg (~351 and 38 mg/g oil, respectively) and lowest in Pidan (~175 and 28 mg/g oil, respectively). On the other hand, fatty acid compositions of all yolk lipids were consistent, with oleic, palmitic and linoleic acids as the three most abundant fatty acids. Acid and thiobarbituric acid values were generally higher for lipids in processed eggs than those in fresh eggs.

*1.3.3.2.1. Triglycerides*

Yolk lipid contains about 65% triacylglycerols or triglycerides (TG). The saturated palmitic acids and stearic acids constitute 30–38% of the fatty acids in yolk lipid, while monounsaturated (primarily oleic acid) and polyunsaturated (including linoleic and arachidonic acid) fatty acids each represent another one-third of the fatty acids (Table 1.7). Position 1 of the yolk TG is occupied predominantly by saturated palmitic acid, while position 2 contains the unsaturated oleic and linoleic acids. Position 3 includes both saturated (palmitic and stearic) and unsaturated (oleic) fatty acids (Juneja 1997).

An and Kang (1999) investigated effects of dietary fat sources with omega-3 or omega-6 PUFA on lipid metabolism and fatty acid composition of egg yolk in laying hens, and reported no effect on the lipid fraction contents in the egg yolk. Du et al. (1999) indicated that the amount of CLA incorporated into total lipid, TG, phosphatidyl choline (PC), and phosphatidylethanolamine (PE) of egg yolk was proportional to the levels of CLA in the diet; more CLA was incorporated in TG than in PC and PE. The incorporation rates of different CLA isomers into different classes of lipids also were significantly different.

Lee et al. (2002b) investigated the influence of dietary tung oil, containing a high level of α-eleostearic acid (*cis*-9,*trans*-11,*trans*-13-octadecatrienoic acid, EA) on growth, egg production, and lipid and fatty acid compositions in tissues and egg yolks of laying hens in White Leghorn hens. α-EA was not deposited in the tissues and egg yolk of hens fed tung oil, but conjugated linoleic acid (CLA) was detected in all tissues and egg yolks. These results suggested that dietary tung oil affected the lipid metabolism of laying hens and could modify the lipid and fatty acid composition in tissues and eggs.

Ginzberg et al. (2000) reported that in chickens fed with biomass of the red microalga *Porphyridium* sp, linoleic acid and arachidonic acid levels increased by 29% and 24%, respectively. In addition to PUFA such as arachidonic acid and EPA, about 70% of the algal dry weight is composed of a unique combination of soluble sulfated polysaccharide. Wang and Pan (2003) indicated that inclusion of red fungus rice in chicken feed reduced egg triglyceride level and LDL concentration.

Brady et al. (2003) reported that hen egg yolk contained significant antibacterial activity, which was associated with the release of free fatty acids. Chloroform–methanol extraction on egg yolk demonstrated the activity to be lipoprotein-bound before enzymatic digestion and associated with the lipid-soluble chloroform phase afterward. Acetone extraction yielded a fraction containing 97% TG, which on treatment with pancreatin showed high antibacterial activity against *Streptococcus mutans*. Both oleic and linoleic acid were found to inhibit growth of *S. mutans*.

*1.3.3.2.2. Phospholipids*

The major components of egg yolk phospholipids (PL) are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which make up ~81% and 12% of egg yolk lecithin; lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and sphingomyelins are also components of yolk PL. Polyunsaturated fatty acids are especially concentrated in the *sn*-2 position, while saturated fatty acids are found in the *sn*-1 position of yolk phospholipids (Juneja 1997). The major fatty acids in egg PC are palmitic, oleic, stearic, and linoleic acids, representing 32%, 26%, 16%, and 13%, respectively; arachidonic and docosahexaenoic acids (4.8% and 4%, respectively) are also present in significant amounts (Juneja 1997).

Yolk phospholipid contents, expressed in relation to weight of egg oil or whole egg, was reported to be positively related ($p < .05$) to hen age (Lin and

Lee 1996). Eggs from hens receiving low-dose chitosan treatment contained 1.8-fold increase in yolk phospholipids level (Vrzhesinskaya et al. 2005).

Kivini et al. (2004) studied the influence of oil-supplemented feeds (containing 15% vegetable-based or fish oils) on the concentration of phospholipids and their composition in hen eggs. Although the total phospholipid contents and proportions of PC, PE, and sphingomyelin were similar for all feeding groups; supplemented feeds had a statistically significant ($p < .05$) effect on the fatty acid composition of phosphatidylcholines. Supplements decreased the proportion of saturated fatty acids in total fat, but not in the phospholipids.

Shimizu et al. (2001) investigated effects of feeding dietary fish oil to hens on the fatty acid composition of eggs. Variation in fatty acid composition in egg yolks was found in the acyl groups of PC and PE, rather than in TG. Results showed that supplementing the diets of hens with fish oil altered essential fatty acid composition, in particular by increasing DHA and decreasing arachidonic acid in egg yolk phospholipids.

Nakane et al. (2001) reported growth factor-like lipids in hen egg yolk and white, which were associated with high amounts of lysophosphatidic acid (acyl LPA) and small amounts of lysoplasmanic acid (alkyl LPA). The levels of acyl LPA in hen egg yolk (44.23 nmol/g tissue) and white (8.81 nmol/g tissue) were on the same order as or higher than the levels of acyl LPA required to elicit biological responses in various animal tissues. Egg yolk acyl LPA contained predominantly saturated fatty acids as the acyl moiety, whereas egg white acyl LPA contained primarily PUFA.

Many studies have been conducted on methods for extraction and separation of phospholipids or lecithins from egg [e.g., see Kim et al. (1995), Nielsen (2001), Yoon and Kim (2002), Palacios and Wang (2005a, 2005b)], as well as preparation of lysolecithin by the enzymatic action of phospholipase A2 (PLA2), including immobilized PLA2 (Kim et al. 2001a). In addition to providing sources of purified phospholipids for basic research, these methods have been established to meet the demand to produce purified egg lecithin for pharmaceutical, nutraceutical, and food applications. Examples of properties of yolk phospholipids with potential industrial applications as nutraceuticals and functional food ingredients include antioxidative activity (Sugino et al. 1997a) and inhibition of cholesterol absorption (Jiang et al. 2001).

*1.3.3.2.3. Sphingomyelins*

Sphingomyelins are present as a minor component in egg yolk lipid, constituting only ~2% of yolk phospholipid. The major component of egg yolk sphingomyelins are palmitosylsphingosines, *N*-acetyl-*O*-trimethylsilyl derivatives of long-chain base residues of natural sphingomyelin (Olsson et al. 1997).

Sphingomyelins are components of animal cell membranes, and their interactions with other membrane constituents including phospholipids and cholesterol are of considerable interest. Lindblom et al. (2006) compared the translational dynamics for bilayers with various mixtures of 1,2-

#### *5.2.2.2. Phosvitin*

Phosvitin is a glycoprotein containing a higher phosphorus content, (nearly 10%) and is considered one of the most highly phosphorylated natural (i.e., nonsynthetic) proteins (Ito and Fujii 1962). The special feature in its amino acid composition is that 30–50% of amino acids are serine, wherein most serine residues are phosphorylated to form phosphoserines. Phosvitin also combines with many bivalent metal ions; for instance, 95% of the Fe ions in an egg are present in the egg yolk, which helps the phosvitin combine with other species via its strong binding ability, and this eventually inhibits the reverse distribution of Fe ions in the living body. The strong binding ability of Fe with phosvitin also exhibits an antibacterial effect in Fe-deficient bacteria (Sattar Khan et al. 2000; Choi et al. 2004) and imparts an antioxidation function to the phospholipids (Lu and Baker 1986). Phosvitin and its enzymatic digests provide protection against iron-catalyzed hydroxyl radical formation and protect DNA against oxidative damage induced by Fe(II) and peroxide (Ishikawa et al. 2004). Therefore, phosvitin may be useful for the prevention of iron-mediated oxidative stress-related diseases, such as colorectal cancer (Ishikawa et al. 2004).

Several molecular species of phosvitin of different molecular weights are reported in the literature on their phosphoric acid and sugar contents. There are five known types of phosvitin: B, C, E1, E2, and F with molecular weights of 40, 33, 18, 15, and 13 kDa, respectively; phosvitins B and C are extensively studied major components (Wallance and Morgan 1986). Phosvitin is usually derived from serum vitellogenin (types I, II and III), whereas phosvitins C and F are derived from vitellogenin I. While phosvitin B is derived from vitellogenin II, Wallance and Morgan (1986) reported that phosvitins E1 and E2 could be formed from vitellogenin III. Phosphorylation of phosvitin occured earlier, prior to the secretion of vitellogenin from the liver. A calcium absorption facilitatory effect of phosvitin-origin phosphopeptide was also described. Phosvitin phosphopeptides derived via tryptic hydrolysis followed by partial alkaline dephosphorylation have been reported to enhance calcium-binding capacity and inhibit insoluble calcium phosphate formation (Jiang and Mine 2000, 2001).

#### *5.2.2.3. LDL in Granules*

Although LDL occurs in a limited quantity, it also exists in granules. Structurally, it seems to be identical to those of plasma LDLs derived from the protein and similar in constitution to the lipids.

## 5.3. YOLK LIPIDS

The lipid content of the egg is mainly present in the egg yolk. The white consists of 88% water, 11% protein, and a small amount of carbohydrate and mineral. On the other hand, the weight proportions for egg yolk are about one-half water (48%), one-third lipids (34%), and one-sixth proteins (17%),

as shown in Figure 5.1 (Li-Chan et al. 1995). All portions of the egg yolk lipid exist as a lipoprotein combined with a protein. The nutritients provided by egg yolk are sufficient to facilitate the birth and growth of a chick, and the lipids also exist in duplex quantities of the protein. The egg yolk lipid facilitates the hatching process of the egg and provides important ingredients for the cell membrane, which is a basic life-sustaining atomic structure and also the main energy source for the chick. Yolk lipids in precise quantities, are also crucial in development of the chick's cerebral component, which is an aggregate of nerve cells.

In this section, we briefly introduce the components of egg yolk lipid and their characteristics and potential health benefits. The latest information on a cholesterol study is also discussed, revealing that egg cholesterol is indeed not a risk factor for heart disease.

### 5.3.1. Components of Egg Yolk Lipid

In general, the lipid content total egg yolk weight is nearly 30%; thus the 20 g of yolk in the average egg contains approximately 6 g of lipids. Neutral lipid (65%), the phospholipids (30%), and cholesterol (4%) are the major components of egg yolk lipids (Figs. 5.3 and 5.4) (Li-Chan et al. 1995). The role of neutral lipid in the hatching process is mainly as an energy-supplying source, while the phospholipids and cholesterol are important ingredients promoting the formation of somatic cell structures and the cell membrane of cranial nerve cells (phospholipid bilayer) of the chick. Thus, the most important function of the egg yolk lipid is to provide a high content of phospholipids and cholesterol as the constituent materials in the cell membrane (Juneja 1997).

Egg yolk phospholipids, also known collectively as *yolk lecithin*, consist of 84% phosphatidylcholine (PC), 12% phosphatidylethanolamine (PE), 2% sphingomyelin, and 2% lysophosphatidylcholine (LPC) and other minor components. A systematic comparison of soybean-derived phosphatides (soy leci-

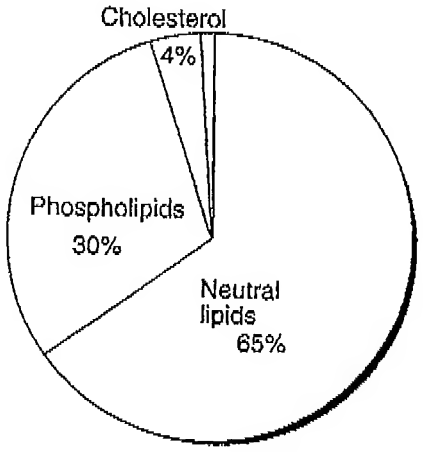


**Figure 5.3.** Composition of egg yolk lipids.

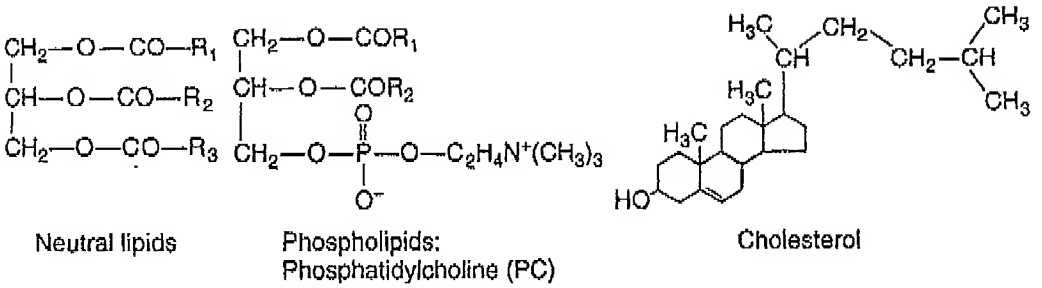


**Figure 5.4.** Basic structure of egg yolk lipids.

**TABLE 5.2. Comparison of Phospholipids**

| Name of Phospholopid | Abbreviation | Egg Yolk (%) | Soybean (%) |
|---|---|---|---|
| Phosphatidylcholine | PC | 84.30 | 33.00 |
| Phosphatidylethanolamine | PE | 11.90 | 14.10 |
| Phosphatidylinositol | PI | | 16.80 |
| Phosphatidic acid | PA | | 6.40 |
| Sphingomyelin | SM | 1.90 | |
| Lysophosphatidylcholine | LPC | 1.90 | 0.90 |
| Others | | | 28.80 |

thin) is presented in Table 5.2 (Weiner et al. 1989). Because of their high phosphatidylcholine content, egg yolk lipids are exceptionally promising for applications in biomedical as well as cosmetics industries.

Typically, in a 100-g egg yolk fraction, lipid constitutes ~30 g, which consists of ~25.4 g of fatty acids. The typical fatty acid composition of egg yolk lipid is described in Table 5.3. The major fatty acids are oleic acid (OA; 43.6%), palmitic acid (PA; 25.1%), linoleic acid (LA; 13.4%), stearic acid (SA; 8.6%), palmitoleic acid (PcA; 3.6%), docosahexaenoic acid (DHA; 1.8%), and arachidonic acid (AA; 1.7%). In addition, either α-linolenic acid (α-LA) or eicosapentaenoic acid (EPA) exists in fatty acid (Tesedo et al. 2006).

Regarding the composition of egg yolk fatty acid, the most of the oleic acid content is reported to reduce serum cholesterol values. Also, while the DHA and AA found in sufficient quantities in egg yolk lipids are indispensable for development of the human newborn's brain and retina, the mother's milk is an additional source of such nutrients (Makrides et al. 2002).

### 5.3.2. Qualitative Value of the Yolk Lipid

Fatty acids can be classified in terms of qualitative values. The total volume of egg yolk lipids (25.4 g), containing saturated fatty acid (8.7 g; 34 vol%), monounsaturated fatty acid (12.2 g; 48 vol%), and polyunsaturated fatty acid (4.5 g; 18 vol%) have been estimated. A qualitative estimation of these values in egg yolk lipid revealed a polyunsaturated fatty acid:saturated fatty acid (P/S) ratio